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1 **An experimental test of state-behaviour feedbacks: gizzard mass and foraging behaviour in red**
2 **knots**

3

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11

12 **Running title:** Experimental test of state-behaviour feedbacks

13 Summary

- 14 1. Animals frequently exhibit consistent among-individual differences in behavioural and
15 physiological traits that are inherently flexible. Why should individuals differ consistently in their
16 expression of labile traits? Recently, positive feedbacks between state and behaviour have been
17 proposed as a possible explanation for the maintenance of consistent among-individual
18 differences in both state and behaviour. If state affects behaviour, and behaviour reciprocally
19 affects state, then differences in either state or behaviour that arise among-individuals even by
20 chance could be maintained over extended periods of time.
- 21 2. We tested for positive feedbacks experimentally using wild-caught red knots (*Calidris canutus*
22 *islandica*). In the wild, knots exhibit consistent among-individual differences in digestive
23 physiology (the mass of the muscular part of the stomach, the gizzard) and foraging behaviour
24 (diet), two inherently labile traits.
- 25 3. Experimentally manipulated diet quality had a large effect on gizzard mass. Experimentally
26 manipulated gizzard mass reciprocally influenced total food eaten during *ad libitum* trials.
- 27 4. The effect of gizzard mass on diet choice, though in the predicted direction, was not statistically
28 significant. Individuals exhibited consistent differences in foraging behaviour of unknown origin
29 independent of current gizzard mass, as well as large residual unexplained variance in foraging
30 behaviour. These two sources of variation in foraging behaviour overruled the gizzard mass-
31 dependent foraging behaviour and hence eroded the treatment-related differences in gizzard
32 mass.
- 33 5. We conclude that positive feedbacks between diet choice and gizzard mass play at best a limited
34 role in maintaining among-individual variation in gizzard mass in red knots. Furthermore, we
35 suggest that many models of state-behaviour feedbacks likely overestimate their potential
36 importance in maintaining long-term among-individual variation in labile traits because most
37 models of state-behaviour feedbacks fail to account for the effects of additional factors that may
38 act to disrupt the feedback loops.

39 6. The among-individual differences in diet choice observed during solitary foraging trials eroded the
40 consistent among-individual differences in gizzard mass observed following periods of staple diet
41 treatments in which knots foraged in social groups. Social foraging interactions may play an
42 important role determining the expression of foraging behaviours such as intake rate that in turn
43 influence gizzard mass. Further studies are needed to experimentally test the role of social
44 interactions as a mechanism generating consistent among-individual differences in foraging
45 behaviours and gizzard mass.

46

47 **Keywords:** animal personality, diet choice, digestive constraints, gizzard mass, physiological
48 plasticity, state-dependent behaviour

49 **Introduction**

50 Behavioural and physiological traits are often inherently flexible and responsive to changes in the
51 environment (Piersma & van Gils 2011). However, within a population, individuals frequently differ
52 consistently in their expression of these traits, referred to as ‘individual specialization’, ‘consistent
53 among-individual differences’, or ‘niche differentiation’ (Bolnick *et al.* 2003; Araújo, Bolnick &
54 Layman 2011; Dall *et al.* 2012; Sih *et al.* 2015). Why should individuals differ consistently in their
55 expression of labile traits? Recently, positive feedbacks between state and behaviour have been
56 proposed as a possible explanation for the maintenance of consistent among-individual differences
57 in both behaviour and state (Sih & Bell 2008; Wolf, van Doorn & Weissing 2008; Luttbeg & Sih 2010;
58 Sih *et al.* 2015). If state affects behaviour, and if behaviour simultaneously affects state, then, when
59 among-individual differences in either state or behaviour arise, even if by chance (e.g. stochastic
60 processes), among-individual differences in both state and behaviour could be maintained over
61 extended periods of time.

62 Several theoretical models have demonstrated the potential importance of positive
63 feedbacks under a range of scenarios (reviewed in Sih *et al.* 2015). For example, if foragers become
64 less vulnerable to predators as they grow larger due to state-dependent safety, then larger
65 individuals may be expected to be relatively more willing to forage under elevated predation danger.
66 At the same time, by virtue of their increased willingness to forage under higher levels of predation
67 danger, larger individuals may acquire the additional resources necessary to maintain or even grow
68 in body size (Luttbeg & Sih 2010). When such positive feedbacks are present (i.e. the effect of
69 behaviour on state and the effect of state on behaviour act to reinforce one another), small
70 stochastic among-individual differences in either state or behaviour can be maintained over
71 extended periods of time. Despite growing interest in the role of state-behaviour feedbacks in
72 maintaining among-individual variation in labile traits, experimental tests of state-behaviour
73 feedbacks are lacking (Sih *et al.* 2015).

74 Here we report on an experiment conducted with wild-caught red knots (*Calidris canutus*
75 *islandica*) testing for feedbacks between diet choice and gizzard mass. Red knots are long-distance
76 migrating shorebirds that breed in the High Arctic and forage on hard-shelled molluscs in coastal
77 estuaries during the rest of the year (Piersma 2007; Buehler & Piersma 2008). Red knots ingest their
78 invertebrate diet whole and crush hard-shelled prey in their muscular gizzards. Gizzard mass is
79 remarkably flexible; knots can adjust their gizzard mass to seasonal changes in energy demands
80 (Piersma 2002; Piersma & van Gils 2011), and as a function of the availability of prey of different
81 digestive qualities (Dekinga *et al.* 2001). Gizzard mass for knots wintering in the Dutch Wadden Sea
82 range from circa 2 to 12 g (van Gils *et al.* 2005). Intriguingly, gizzard mass predicts patterns of habitat
83 use over the course of weeks (Oudman *et al.* 2016) up to many months (Bijleveld *et al.* 2014), which
84 suggests that among-individual differences in gizzard mass are stable over extended periods of time.
85 Given that gizzard mass is a labile trait with the potential to change in size several-fold over the
86 course of days (Dekinga *et al.* 2001), why do free-living knots exhibit consistent among-individual
87 variation in gizzard mass?

88 One obvious explanation for consistent among-individual differences in gizzard mass
89 variation is variation in overall body size: larger-bodied birds may have larger gizzards. Indeed,
90 analyses of over 1000 gizzard mass measurements taken on red knots captured in the Dutch Wadden
91 Sea during the overwintering period (October through February) show significant correlations with
92 measures of structural body size such as wing chord, bill length, tarsus (Dekinga & Piersma,
93 unpublished data, see Appendix S1 in Supporting Information). However, structural body size
94 explains a relatively small proportion of the total variation in gizzard mass (< 2%), which suggests that
95 some other factor(s) are the main drivers of variation in gizzard mass.

96 Positive feedbacks between gizzard mass and diet choice offer another potential explanation
97 for consistent among-individual differences in gizzard mass (Piersma, Koolhaas & Dekinga 1993;
98 Piersma *et al.* 1999). Earlier work has already demonstrated that the digestive quality of the diet
99 strongly affects gizzard mass (Dekinga *et al.* 2001; Bijleveld *et al.* 2014). When the diet of knots is

100 experimentally manipulated, knots fed a lower quality diet with a low ratio of digestible to
101 indigestible components develop larger gizzards (e.g. Dekinga *et al.* 2001; Bijleveld *et al.* 2014). Food
102 processing rates are faster with increasing gizzard mass (van Gils *et al.* 2003), consequently, as
103 gizzard mass increases, digestive constraints decrease, where digestive constraint refers to the
104 extent to which intake rate is limited by digestive processing rate. Digestive rate maximizing models
105 predict that diet breadth should increase as digestive constraints decrease (Hirakawa 1995; 1997);
106 because as gizzard mass increases, knots should be relatively more willing to consume low quality
107 prey in addition to high quality prey. A greater willingness to consume low quality prey items by
108 some individuals would reinforce variation in gizzard mass by facilitating the maintenance of larger
109 gizzards in these individuals. Conversely, if individuals with small gizzards are less willing to accept
110 low quality prey, their diet selection would prevent them from developing larger gizzards. Thus,
111 variation in gizzard mass and diet choice arising from chance variation in encounters with high versus
112 low quality prey could theoretically be maintained over extended periods of time via positive
113 feedbacks between digestive physiology and foraging behaviour (Bijleveld *et al.* 2014).

114 We tested for positive feedbacks between gizzard mass and diet choice in a laboratory
115 experiment using wild-caught red knots and evaluated whether such feedbacks are sufficient to
116 account for observed differences in gizzard mass and diet choice in free-living knots. First, we
117 manipulated diet quality and recorded the consequences of diet manipulations on gizzard mass.
118 Second, we tested whether experimentally manipulated gizzard mass influenced diet choice.
119 Specifically, we tested the prediction that knots would show a greater willingness to accept low
120 quality prey items in their diet if their gizzards were experimentally enlarged from long-term
121 exposure to a low digestive quality diet.

122

123 **Methods**

124 ***Study subjects***

125 Twenty red knots were captured using mist nests on the mudflats of Richel (53°16'57"N, 05°23'82"E)
126 in the Wadden Sea, The Netherlands on 27 January 2015. Prior to laboratory experiments, birds were
127 housed in aviaries at the NIOZ Royal Netherlands Institute for Sea Research, in 2 flocks of 10 birds.
128 Aviary dimensions were 3.85 x 1.85 m and 2.40 m high. Temperature and photoperiod matched
129 prevailing ambient conditions. Aviary floors were kept wet with a constant stream of filtered sea
130 water and a tray of running freshwater was always available for drinking and bathing. Birds were
131 maintained on a diet of protein-rich trout-feed pellets (Produits Trouw, Vervins, France) that was
132 available *ad libitum*. Birds were handled each week to assess overall health and to allow aviaries to
133 be cleaned. Prior to experiments, two birds died of unknown causes. Three additional birds were
134 excluded from the experiments; two because they developed small breast wounds which precluded
135 measurements of gizzards during the experiments (see below for description of gizzard
136 measurements) and one because it had low body mass in the two weeks preceding the experiment
137 and therefore we chose not to include it in a protocol that involved regular periods of food
138 deprivation (see below). Each of these three birds improved in condition over the subsequent weeks
139 and was later released. Thus, our experiments were carried out with N = 15 birds, all of which were in
140 good health and released at the end of the experiment.

141 ***Gizzard mass manipulations***

142 We used a within-subjects study design in which we manipulated gizzard mass twice in each bird,
143 hereafter "large gizzard" and "small gizzard" treatments. The order of treatment was randomized:
144 half of birds received the large gizzard treatment first (N = 8) and half received the small gizzard
145 treatment first (N=7). Gizzard mass was manipulated by changing the digestive quality of their staple
146 diet. To induce small gizzards, knots were fed a high digestive quality diet of blue mussels, *Mytilus*
147 *edulis*, whose shells were opened by briefly submerging the mussels in boiling water (see Bijleveld *et*

148 *al.* 2014). When presented with mussels with open shells, knots consume only the flesh of the
149 mussel, thereby removing the need to process shells in their gizzard. Previous experiments with
150 knots have shown that a diet of open mussels induces an average gizzard mass of approximately 2 to
151 3 g (Bijleveld *et al.* 2014).

152 To induce large gizzards, knots were fed a low digestive quality diet of thawed mudsnails,
153 *Hydrobia ulvae*, a marine gastropod snail. Freezing *Hydrobia* does not separate the mollusc flesh
154 from the shell, and therefore, knots consumed *Hydrobia* whole and needed to process large volumes
155 of *Hydrobia* shells in their gizzards. To minimize variance in digestive quality over the course of the
156 experiments, all *Hydrobia* used in these experiments were collected on a single sampling occasion
157 (12 March 2015) in the Wadden Sea near the Afsluitdijk (52°58'07"N, 5°06'19"E). Previous
158 experiments with knots have shown that a staple diet of *Hydrobia* during the same time of year
159 (spring) induces a gizzard mass of roughly 6 to 7 g (Vézina, DeKinga & Piersma 2011). Therefore, we
160 expected our two gizzard mass manipulations to result in approximately 2-fold variation in gizzard
161 mass.

162 We chose to use *Hydrobia* rather than closed mussels to induce large gizzards. Using closed
163 mussels for the "large gizzard" treatment would have had the advantage of being the same food type
164 as used in the "small gizzard" treatment, thereby controlling for treatment related differences in the
165 nutritional quality of food offered during the experiments. However, closed mussels can only be
166 stored for up to 1 week, and therefore, would have had to have been collected on numerous
167 occasions over the course of the experiments. Given that the digestive quality of mussels changes
168 seasonally as mussels grow (Dare & Edwards 1975), closed mussels would not have provided a
169 standard gizzard mass manipulation over the 8 week duration of our laboratory experiments. In
170 contrast, the *Hydrobia* used during the experiments were collected in a single day, and were
171 therefore of uniform digestive quality throughout the experiments. Furthermore, in earlier
172 experiments, knots fed staple diets of either *Hydrobia* (e.g., Vézina *et al.* 2006) or mussels (e.g.,

173 Bijleveld, Folmer & Piersma 2012) over the course of several months were healthy and had good
174 body condition, indicating that each of these two food types meet the nutritional needs of knots.

175 In captivity, knots adjust gizzard mass to changes in diet within approximately 1 week
176 (Dekinga *et al.* 2001). We allowed birds 3 weeks to adjust their gizzard mass to staple diets before
177 carrying out diet choice experiments which lasted 1 week. Each bird was subjected to both diet
178 treatments, in random order. Thus, the entire experiment lasted approximately 2 months.

179 ***Gizzard measurements***

180 Gizzard mass was measured by AD using an ultrasound scanner (model Aquilla, Pie Medical Benelux,
181 Maastricht, The Netherlands). Birds were not anesthetized for the procedure, which takes
182 approximately 3 to 5 minutes per individual, and knots remained calm during handling. Prior to the
183 procedure, animals are fasted for at least 1 hour (range 1 hr to 3 hours) to ensure that their gizzards
184 were empty and therefore, that variation in gizzard measurements reflects differences in the organ
185 size as opposed to differences the amount of organ contents. During the procedure, an individual is
186 placed on its back on the lap of the observer (AD) and ultrasonic gel is smeared on its belly to couple
187 the probe to the surface of the animal. The width and height of the gizzard are measured by placing
188 the probe transversely on the belly of the bird at a 45° angle just below the rib cage. For more
189 detailed descriptions of the procedure, see Dietz *et al.* (1999) and Dekinga *et al.* (2001). In each
190 measurement session, gizzard height (cm) and gizzard width (cm) were measured twice for each bird.
191 Subsequently, the average height and average width were used to estimate gizzard mass in grams
192 using the following equation:

$$193 \quad \text{gizzard mass (g)} = -1.09 + 3.78 \times (\text{height}_{\text{average}} \times \text{width}_{\text{average}})$$

194 The equation and coefficients were estimated from a regression of similarly obtained gizzard
195 measurements against fresh gizzard mass (in grams, excluding any contents) from dead birds that
196 could be dissected ($r = 0.92$, $p < 0.01$, $N = 27$) (Bijleveld *et al.* 2014). Gizzard mass was measured
197 within 24-hr of capture. This measure reflects gizzard mass of free-living knots (hereafter, field

198 gizzard). Additionally, gizzard mass was measured on 4 occasions for each bird over the course of the
199 experiments: before the start of each series of diet choice experiments (n = 2), and at the end of each
200 series of diet choice experiments (n = 2). The gizzard observer (AD) was blind to the gizzard mass
201 manipulation of the birds as well as to their foraging behaviour during diet choice trials.

202 ***Diet choice experiments***

203 Baltic tellins, *Macoma balthica*, a small marine bivalve of different size classes (see details below and
204 in Appendix S2 and Table S1) were used during foraging trials to test the prediction that knots alter
205 prey choice as a function of their digestive constraints. We used *Macoma* ranging in size from 11 to
206 16 mm during foraging trials, which is within the range of sizes of *Macoma* in the natural diets of
207 knots (Zwarts & Blomert 1992; Dekinga & Piersma 1993). On days where foraging trials were carried
208 out, food was removed from holding aviaries at 6:00. Foraging trials were carried out between 12:00
209 and 18:00. Thus, birds experienced a minimum deprivation of 6:00 prior to trials, which is equivalent
210 to non-feeding times experienced by free-living knots when their foraging grounds are inundated
211 during high tides. Outside of experimental periods (18:00 to 6:00), birds had *ad libitum* access to
212 their staple diets (either *Hydrobia* or open mussels). For each bird and each gizzard mass
213 manipulation, we attempted 3 replicates of the foraging trials (see details below). Between 4 and 11
214 birds were tested each day, and it took 7 days of tests to complete 3 replicates of the foraging trials
215 for all 15 birds during each of the two experimental blocks.

216 *Phase I: Ad libitum trials*

217 Knots were tested individually in diet choice experiments, which were carried out in two steps. Tests
218 began by catching a randomly pre-determined focal individual from their group aviary, weighing the
219 individual, then placing the individual in a testing aviary with identical dimensions to the group
220 holding aviaries. Next, 50 intermediately sized *Macoma* (13 - 14 mm) were evenly spaced on a tray
221 that was placed in the aviary, and the focal bird was allowed to feed for 40 min. The trays were 60 x
222 40 x 5 cm (width x length x depth), and were the same type of trays in which birds had *ad libitum*

223 access to food outside of the experiments and were therefore familiar to birds. At the end of the
224 trial, the tray was removed and the number of *Macoma* eaten was recorded. The first phase of the
225 experiment served two purposes: (1) it allowed us to ensure that birds were motivated to feed
226 before testing their diet preferences, and (2) it aimed to ensure that birds entering the diet choice
227 phase of the experiment (phase 2) were digestively constrained (i.e. did not have empty gizzards).
228 Birds that did not eat any *Macoma* during *the ad libitum* trials were neither motivated nor
229 constrained (their gizzards were empty), and they were returned to their group aviary and the next
230 focal individual was taken. In total, there were 3 individuals that never ate during the trials, and thus
231 we obtained diet choice data for a total of 12 individuals. We chose not to increase the duration of
232 the food deprivation as a means to increase feeding motivation as we wanted to avoid influencing
233 gizzard physiology with unnaturally long periods of food deprivation. However, the probability of
234 feeding during the *ad libitum* trials was not related to the gizzard treatment (see Results).

235 Phase II: Diet choice

236 Any birds that ate ≥ 1 *Macoma* were carried forward into the second phase of the experiment on diet
237 choice. The diet choice experiment consisted of 20 sequential presentations of two prey types: high
238 digestive quality *Macoma* (10.5 – 12.5 mm size class) and low digestive quality *Macoma* (14.5 – 16.5
239 mm size class). Size classes of *Macoma* were selected on the basis of ratio of flesh to shell mass
240 (digestive quality) determined in a subsample of *Macoma* prior to the start of the experiments (see
241 Appendix S2 for details regarding estimates of digestive quality). Prey were presented in alternating
242 order, always beginning with the high quality prey item, similar to the protocol outlined in Krebs *et*
243 *al.* (1977). We created an experimental scenario where searching time for each prey type was fixed
244 at 2 min by presenting the next prey item 1 minute after a bird entered the food tray. Birds only
245 entered the food tray for feeding and for this reason birds that entered the tray were deemed to be
246 searching for food. The tray was removed after 1 min independent of whether or not the prey item it
247 contained was consumed. The tray was immediately replaced with a new tray containing the next
248 prey item to be presented. By presenting the subsequent prey item 1 min after the bird entered the

249 tray, handling time was effectively zero, as it had no effect on the interval at which prey were
250 encountered. Based on the realized effect of the gizzard mass manipulations and the estimated
251 energy and ballast content of *Macoma*, we predicted that knots with large gizzards should accept
252 both prey types, while knots with small gizzards should only accept high quality *Macoma* (see
253 Appendix S3 for calculations). Tests ended before completing 20 presentations if the time elapsed
254 since the start of the test was greater than 90 min or if the focal bird took greater than 20 minutes to
255 approach the food tray after it was presented, as the bird was assumed to no longer be motivated to
256 feed at this point.

257 **Statistical analyses**

258 First, we confirmed that gizzard mass manipulations were effective. Gizzard mass following each
259 series of 3 week staple diet manipulations was modelled as a function of the treatment (small gizzard
260 or large gizzard), and individual identity was fitted as a random effect. Gizzard mass was modelled
261 with Gaussian errors. We also tested for a possible effect of treatment order (both alone and in
262 interaction with current treatment). There was no support for such effects (results not shown), and
263 therefore, treatment order was not considered further.

264 Next, we tested whether the gizzard mass manipulation predicted the probability of
265 participating in foraging trials. We modelled the decision to participate in a given replicate attempt
266 (Yes = 1 (n= 50); No = 0 (n= 40)) as a function of the gizzard treatment. Individual identity was fitted
267 as a random effect. The decision to participate was modelled with binomial errors.

268 For birds that did participate in the trials, we tested whether the gizzard mass manipulation
269 resulted in differences in intake rate of *Macoma* during *ad libitum* trials, which we assume reflects
270 variation in digestive capacity. We modelled the number of *Macoma* eaten during *ad libitum* trials as
271 a function of gizzard mass treatment. We also included individual-treatment specific replicate as a
272 fixed effect to account for the possibility that gizzard mass, and hence prey ingestion, changed across
273 successive trials. Individual-treatment specific replicates were left-zeroed (i.e. the first replicate in

274 which a bird consumed *Macoma* was coded as zero, the second was coded as 1, and the third was
275 coded as 2) so that the model estimates for each treatment level (large gizzard or small gizzard)
276 provided an estimate of the behaviour of birds during the first trial in which they participated.
277 Individual identity was fitted as a random effect. The number of *Macoma* eaten for birds that did
278 participate in the trials (i.e. that ingested ≥ 1 *Macoma*) was normally distributed, and models were
279 constructed with a Gaussian error distribution.

280 We also tested whether our experimental manipulations of gizzard mass resulted in
281 differences in prey acceptance. We modelled the decision to accept (1) or reject (0) either high or
282 low quality *Macoma* as a function of gizzard mass treatment (small gizzard or large gizzard). Again,
283 we modelled changes in acceptance across successive replicates to account for possible changes in
284 prey choice over time. Individual identity was fitted as a random effect, and models were
285 constructed with a binomial error distribution.

286 We also investigated whether any observed differences in acceptance probabilities reflected
287 differences in the digestive quality of realized diets due differences in the relative acceptance of high
288 versus low quality *Macoma*, rather than differences in feeding rate or feeding motivation caused by
289 differences in the probability of accepting *Macoma* overall, but no differences in the relative
290 acceptance of high versus low quality prey. We repeated the above analyses using the number of low
291 digestive quality *Macoma* versus high digestive quality *Macoma* accepted by foraging birds as
292 response variables (using the 'cbind' function). Gizzard treatment and trial replicate were included as
293 fixed effects, and individual identity was fitted as a random effect. The model was constructed with a
294 binomial error structure.

295 The above analyses revealed changes in prey acceptance across successive replicates. We
296 therefore tested whether behavior during the foraging trials resulted in changes in gizzard mass.
297 Here, we modelled changes in gizzard mass (end gizzard mass – start gizzard mass) as a function of
298 the number of *Macoma* consumed during the foraging trials, experimental treatment (large or small

299 gizzard), and the interaction. Individual ID was included as a random effect, and the model was
300 constructed with Gaussian errors.

301 Analyses of a much larger data set of gizzard mass show that body size explains only a small
302 proportion of the total variance in gizzard mass (<2%, see Appendix S1). Furthermore, our
303 experiments employ a within-subject design with a randomized treatment order, thus providing
304 controls for among-individual differences in body size. Therefore, we did not include structural body
305 size as a covariate in our models. All mixed effects models were constructed in R v.3.1.2 (R Core
306 Team 2014) using the 'lme4' package. We used the 'sim' function of the 'arm' package to simulate
307 values of the posterior distribution of the model parameters. Ninety-five percent credible intervals
308 (CI) around the mean (β) were extracted based on 1000 simulations (Gelman & Hill 2007). We
309 evaluated support for effects based on estimated effects sizes and their 95% credible intervals
310 (Cumming & Finch 2005; Nakagawa & Cuthill 2007). This approach has been advocated to avoid
311 drawing dichotomous conclusions to accept or reject the null hypothesis based on data which can
312 show a continuous range of support (or lack of support) for a given interpretation (Cohen 1990).
313 However, as a reference for readers less familiar with CIs, a 95% CI is roughly equivalent to a $p \leq 0.05$
314 when the null hypothesis is anything outside the bounds of the CI (i.e., no directional prediction), or p
315 ≤ 0.025 with directional predictions (Cumming & Finch 2005); we describe such results as showing
316 'strong-support' for an effect. For estimates that are biased away from zero but with the 95% CIs
317 overlapping zero (up to 15% of CI), we instead use the term 'moderate support'. For estimates
318 centered on zero, we use the term 'no support for an effect' or the term 'support for lack of effect'.
319 Adjusted repeatabilities (i.e. repeatability after correcting for fixed effects) were calculated following
320 Nakagawa and Schielzeth (2010) as $V_{\text{individual}}/(V_{\text{individual}}+V_{\text{residual}})$, where $V_{\text{individual}}$ is the individual
321 variance component and V_{residual} is the residual variance.

322

323 **Results**

324 Manipulations of the digestive quality of the diet had the intended effect on induced gizzard mass.
325 The average gizzard mass following the small gizzard treatment was 3.60 g (95% CI = 3.03, 4.10)
326 versus 6.17 g following the large gizzard treatment (95% CI = 5.62, 6.67). The difference between
327 treatments was significant ($\beta = 2.45$ g, 95% CI = 2.10, 3.08). We also observed significant individual
328 repeatability of gizzard mass ($r = 0.34$, 95% CI = 0.25, 0.71) indicating that, even while adjusting
329 gizzard mass to current staple diets, individual knots with relatively large gizzards in one treatment
330 also had relatively large gizzards in the other treatment (Figure 1).

331 Not all birds participated in each attempted replicate of the foraging trials. However, the
332 probability (log odds ratio) of eating during *ad libitum* trials was not related to treatment (small
333 gizzard: $\beta = 0.20$, 95% CI = -0.76, 1.29; large gizzard: $\beta = 0.39$, 95% CI = -0.82, 1.41; estimated
334 difference: $\beta = 0.04$, 95% CI = -1.05, 0.95). However, the finding that individuals showed high
335 repeatability in their probability of participating in trials independent of current gizzard mass ($r =$
336 0.74 , 95% CI = 0.57, 0.81) suggests the presence of intrinsic among-individual variation in feeding
337 motivation and/or willingness to eat *Macoma* under the conditions of these experiments.

338 For birds that did participate in the *ad libitum* trials, the number of intermediate sized
339 *Macoma* eaten was greater during the large gizzard treatment compared with the small gizzard
340 treatment (Table 1). The difference was significant (estimated difference $\beta = 5.79$, 95% CI = 0.67,
341 11.93). However, this was only true for the first replicate during which they participated, because
342 during the small gizzard treatment, but not during the large gizzard treatment, birds increased the
343 number of *Macoma* eaten over successive *ad libitum* trials (Table 1, Figure 2). The estimated
344 repeatability of *ad libitum* intake was low ($r = 0.05$, 95% CI = 0.02, 0.09), indicating a lack of among-
345 individual differences in the quantity of *Macoma* eaten after controlling for current gizzard mass
346 treatment.

347 During the diet choice trials, knots were more likely to accept high quality *Macoma*
348 compared with low quality *Macoma* (Table 1, Figure 3). This diet preference indicates that knots
349 were clearly able to discriminate between these two categories of *Macoma*. Following the digestive
350 rate maximizing model (Hirakawa 1995; 1997), we predicted that birds would have a 100%
351 acceptance probability for high quality *Macoma* during both gizzard treatments (see Appendix S3 and
352 Figure S1 for calculations of predicted acceptances). Consistent with this prediction, the probability
353 of accepting high quality *Macoma* did not vary as a function of gizzard mass, however, the overall
354 acceptance rate was lower than predicted (~85%, Figure 3).

355 Overall, knots were much less likely to accept low quality *Macoma*. During the small gizzard
356 treatment, knots were more likely to accept low quality *Macoma* than predicted by the DRM (~ 20%
357 observed versus 10% predicted, Figure 3). In contrast, during the large gizzard treatment, knots were
358 less likely to accept low quality *Macoma* than predicted by the DRM (~ 30% observed versus 100%
359 predicted, Figure 3). The estimated difference in the log-likelihood of accepting low quality *Macoma*
360 across gizzard treatments was in the predicted direction ($\beta = 0.35$, 95% CI = -0.31, 1.21); but, the 95%
361 CI of the difference overlapped with zero (13.6% of estimates were < 0). As during *ad libitum* trials,
362 we observed changes in diet choice across successive trials during the small gizzard treatment, but
363 not during the large gizzard treatment (Table 1). Over the course of successive trials, birds from small
364 gizzard treatments increased their probability of accepting both high and low quality *Macoma* (Table
365 1), perhaps because gizzard mass increased over the course of the foraging trials (see below).
366 Nonetheless, knots showed significant among-individual differences in the propensity to accept both
367 high quality ($r = 0.57$, 95% CI = 0.36, 0.68) and low quality ($r = 0.36$, 95% CI = 0.22, 0.50) *Macoma*.

368 The differences in the likelihood of accepting high and low quality *Macoma* translated to
369 differences in realized diet (Table 1). The ratio of low to high quality *Macoma* in the diet tended to be
370 greater during the large gizzard treatment ($\beta = 0.39$, 95% CI = -0.38, 0.99). Again, there was no
371 evidence for changes in the ratio of low to high quality prey across successive replicates in the large
372 gizzard treatment, but the ratio of low to high quality prey increased across successive replicates in

373 the small gizzard treatment ($\beta = 0.46$, 95% CI = 0.08, 0.74). Furthermore, knots showed significant
374 among-individual differences in realized diet quality ($r = 0.10$, 95% CI = 0.03, 0.16), reflecting
375 differences either in the strength of their preference for high quality prey, their ability to discriminate
376 between high and low quality prey, or some combination of the two factors.

377 Knots tended to increase gizzard mass over the course of the diet trials during the small
378 gizzard manipulation ($\beta = 0.65$, 95% CI = -0.37, 1.40), but no such trend was observed during the large
379 gizzard manipulation ($\beta = 0.02$, 95% CI = -0.90, 0.81). Analyses that controlled for the number of
380 *Macoma* eaten during the trials revealed that birds from the large gizzard treatment decreased in
381 gizzard mass when they did not consume any *Macoma* (Table 2). Birds from the small gizzard
382 treatment that consumed no *Macoma* also tended to decrease in gizzard mass, but this was not
383 significant (Table 2). At the same time, the number of *Macoma* eaten during the foraging trials had a
384 positive effect and gizzard mass increased (effect of number of *Macoma* ingestions, Table 2). The
385 effects of ingestions did not differ across gizzard mass treatments ($\beta = -0.014$, 95% CI = -0.035, 0.015)
386 (Figure 4). Changes in gizzard mass over the course of the foraging trials controlling for the number
387 of *Macoma* ingested also showed individual repeatability ($r = 0.43$, 95% CI = 0.25, 0.58), indicating
388 consistent among-individual differences in gizzard plasticity.

389

390 **Discussion**

391 We experimentally tested and quantified the strength of feedbacks between gizzard mass and diet
392 choice in wild-caught red knots to evaluate the potential role of state-behaviour feedbacks in
393 maintaining among-individual variation in gizzard mass. We found some support for positive
394 feedbacks; diet quality had large and significant effects on gizzard mass, and gizzard mass reciprocally
395 affected *ad libitum* intake rates. However, we found only moderate support for a small effect of
396 gizzard mass on diet choice. We also observed several-fold variation in diet choice that was
397 independent of current gizzard mass treatment. The variation resulted in large changes in gizzard

398 mass, which overrode the effects generated via feedbacks between gizzard mass and diet choice.
399 Below, we discuss the implication of our findings for our understanding of consistent among-
400 individual differences in gizzard mass, and in labile traits in general.

401 ***Positive feedbacks between gizzard mass and foraging behaviour***

402 We experimentally tested whether state-behaviour feedbacks could favour the maintenance of
403 consistent among-individual differences in gizzard mass and diet choice in red knots. In agreement
404 with earlier work, we found that experimentally manipulated diet induced large differences in gizzard
405 mass (Dekinga *et al.* 2001; Bijleveld *et al.* 2014). Individuals doubled their gizzard mass during the low
406 digestive quality diet treatment compared with the high digestive quality treatment (Figure 1). We
407 also confirmed that experimentally manipulated gizzard mass influenced digestive constraints. Knots
408 consumed twice as many *Macoma* (12.5-14.5 mm size class) during *ad libitum* foraging trials when
409 they had enlarged gizzards compared to when knots had reduced gizzards. Our findings corroborate
410 earlier work (van Gils *et al.* 2003) showing that larger gizzards have greater digestive processing
411 capacity compared with small gizzards. Following this, we predicted that larger gizzard mass should
412 be associated with lower prey selectivity (Hirakawa 1995; 1997). Additionally, higher intake rates had
413 positive effects on gizzard mass, while low intake rates had negative effects (Figure 4). Thus, gizzard-
414 mass related differences in *ad libitum* intake rates may also feedback to contribute to the
415 maintenance of variation in gizzard mass.

416 As predicted, both the probability of acceptance of low quality *Macoma* and the ratio of low
417 to high quality prey in the diet tended to be greater during the large gizzard manipulation. However,
418 the two effects were weaker than predicted based on digestive constraints estimated following van
419 Gils *et al.* (2003) (see Appendix S3 for derivations of quantitative predictions), and were not
420 statistically significant ($p = 0.13$). The overall higher acceptance of high quality *Macoma* indicates
421 that knots were clearly able to discriminate high and low quality prey (Table 1). Additionally, low
422 quality *Macoma* were consumed to some degree, indicating that other constraints such as external

423 handling time, the ability to swallow *Macoma*, or to mechanically crush shells in their gizzards, did
424 not prevent knots from eating them, and that the low quality *Macoma* used in these experiments
425 were within the acceptable size range for this prey type (Zwarts & Blomert 1992). So why did knots
426 not show stronger adjustment in prey selectivity?

427 The nonsignificant effect of gizzard mass on diet choice may be due in part to the gizzard
428 mass manipulation having had a smaller effect on digestive processing rates than expected.
429 Following van Gils *et al.* (2003), our gizzard mass manipulations were expected to generate five-fold
430 variation in digestive processing rates. However, the observed differences in ballast processing
431 during *ad libitum* foraging trials were much more modest (~2-fold variation, Table 2). The relatively
432 modest change in apparent digestive constraints observed in the present study generates markedly
433 different predictions for gizzard mass related differences in prey choice. The predicted acceptance
434 probabilities for high and low quality *Macoma* become 62% and 0% respectively in the small gizzard
435 treatment, and 100% and 10% respectively in the large gizzard treatment (see Appendix S4 for
436 calculations). Indeed, the observed acceptance rates during foraging trials show much better
437 quantitative agreement with these predictions (Figure 3).

438 Nonetheless, one notable difference remaining between the revised predictions and the
439 observed acceptance rates was that knots exhibited a higher than predicted acceptance of low
440 quality *Macoma* during both gizzard mass treatments (20% and 30% acceptance in small and large
441 gizzard treatment, versus 0% and 10% predicted acceptance, respectively), although the observed
442 difference between treatments matched the predicted difference (10%). Our finding is similar to
443 results in other optimal diet studies (reviewed in Pyke 1984). For example, in one of the first optimal
444 diet studies, Krebs *et al.* (1977) found that great tits (*Parus major*) consistently exploited the less
445 profitable prey type more than predicted under intake rate maximization. Deviations from
446 expectation were interpreted as sampling behaviour, presumably to allow foragers to update their
447 estimates of the profitability of different prey types.

448 ***Positive feedbacks do not explain consistent among-individual differences in gizzard mass***

449 We did find some support of positive feedbacks between gizzard mass and foraging behaviour. The
450 digestive quality of the diet had strong effects on gizzard mass, and gizzard mass reciprocally had
451 strong effects on *ad libitum* intake rates. The effect of gizzard mass on diet choice was less clear: the
452 estimated effect size was in the predicted direction, but the credible intervals overlapped zero.
453 However, even accepting the potential for gizzard mass to influence diet choice in knots, three key
454 observations from our study suggest a limited role for state-behaviour feedbacks in maintaining
455 consistent among-individual differences in gizzard mass. First, feedbacks were strongly asymmetric.
456 Large differences in diet induced large differences in gizzard mass, but large differences in gizzard
457 mass induced at best weak differences in diet choice with predicted and observed effects both ~10%.

458 Second, the majority of variation in foraging behaviour observed, including willingness to
459 participate in *ad libitum* foraging trials, probability of accepting high quality versus low quality
460 *Macoma*, and ratio of low quality to high quality *Macoma* in the diet, was independent of current
461 gizzard mass. Gizzard-independent behaviour had large effects on gizzard mass (Figure 4) and
462 overrode variation generated by feedbacks between gizzard mass and diet choice. Thus, foraging
463 decisions were shaped by more than current gizzard mass. This result is not surprising; it is likely the
464 norm that the expression of labile traits is shaped by multiple pathways simultaneously. However,
465 many models of state-behaviour feedbacks fail to take additional pathways into account (but see
466 Luttbeg & Sih 2010). Our results highlight that when multiple factors act on the expression of labile
467 traits simultaneously, models that do not explicitly take multiple factors into account may
468 overestimate the potential for positive state-behaviour feedbacks to maintain long-term consistency
469 in state and behaviour.

470 Last, we observed significant repeatability of gizzard mass following the staple diet
471 treatments, and experimentally manipulated gizzard mass following both treatments was positively
472 correlated with field gizzard mass (see Figure S2, left panel). Our results demonstrate that neither

473 state-behaviour feedbacks, nor among-individual differences in diet choice, are needed to generate
474 among-individual differences in gizzard mass because the diet manipulations precluded both of these
475 effects as the staple diets were of uniform digestive qualities. Furthermore, the correlations between
476 field gizzard measurements and captive gizzard measurements broke down following the diet choice
477 trials (see Figure S2, right panel). In other words, the opportunity to exert choice over the digestive
478 quality of their diets eroded the among-individual differences in gizzard mass observed between field
479 gizzard measurements and following periods of staple diet. Thus, individual differences in diet
480 preferences, at least as measured in our experiments, are not a primary driver of among-individual
481 variation in gizzard mass in free-living red knots. In line with this view, among-individual differences
482 in foraging behaviour (total intake or diet choice) expressed during foraging trials did not correlate
483 consistently with field gizzard mass (see Figure S3).

484 ***Understanding consistent individual differences in gizzard mass***

485 If neither individual differences in diet choice, nor state-behaviour feedbacks, can account for the
486 long-term maintenance of among-individual differences in gizzard mass, then what factors can?
487 Below we discuss two alternative, non-exclusive, mechanisms that may promote consistent among-
488 individual differences in gizzard mass: environmental conditions during early development and/or
489 social context.

490 Conditions experienced during early development can produce lasting effects on adult
491 phenotypes (Monaghan 2008), and we suggest that among-individual differences in gizzard mass
492 could arise because of differences in early dietary experience. Previous studies have shown that early
493 diet has persistent effects on various aspects of physiology including antioxidant defense (Blount *et al.*
494 *2003*; Noguera *et al.* 2015; Noguera, Monaghan & Metcalfe 2015), metabolic rate (Crisuolo *et al.*
495 *2008*), and digestive physiology (birds: Biviano, Martínez del Rio & Phillips 1993; mammals: Distel *et al.*
496 *1996*; fish: Geurden *et al.* 2007). For example, sheep fed a diet of low digestive quality early in life
497 show lasting differences in digestive efficiency; they are more efficient at extracting nitrogen than

498 sheep that experienced high quality diets early in life (Distel *et al.* 1996). As a result, when the
499 availability of high quality food is low, sheep reared on low digestive quality diets expand the diet to
500 include low quality food more so than sheep reared on high quality diets (Distel *et al.* 1996),
501 consistent with predictions from a digestive rate maximizing model. Knots are likely to experience
502 among-individual differences in average digestive qualities of their diets during early development,
503 for example because they develop in places or at times that differ in prey availability (van Gils *et al.*
504 2016). This in turn may influence their digestive efficiency, similar to findings in sheep. If knots that
505 experience low digestive quality diets develop greater digestive efficiency, then all else being equal,
506 these knots may consistently ingest more food or more food of lower quality, promoting long-term
507 maintenance of larger gizzards relative to knots with lower digestive efficiency.

508 Another, non-exclusive, mechanism that has been suggested previously to generate among-
509 individual differences in labile traits is social interactions (Bell, Hankison & Laskowski 2009; Killen *et*
510 *al.* 2016). Social interactions may shape the foraging opportunities available to knots (e.g.,
511 subordinates have less predictable access to food), which in turn influence gizzard mass. In knots,
512 social dominance is positively correlated with intake rates, even when food patches are non-
513 depleting (Bijleveld, Folmer & Piersma 2012). Given that intake rate has a strong, positive effect on
514 changes in gizzard mass (Figure 4), social foraging interactions may play an important role in
515 generating among-individual variation in gizzard mass. As birds foraged in social groups when they
516 were given the staple diets, but not during the diet choice experiments, this may explain why gizzard
517 mass following each staple diet manipulation was positively correlated with field gizzard mass, but
518 gizzard mass following diet choice experiments was not or was less so (see Figure S2). A lack of
519 competition during the solitary foraging trials may have allowed individuals to express acceptance
520 rates of high versus low quality *Macoma* (Table 1) that they would normally not be able to express in
521 social foraging groups.

522 **Conclusions**

523 We present an experimental test of state-behaviour feedbacks. We found support for positive
524 feedbacks between gizzard mass and intake rates. The effect of gizzard mass on diet choice (selection
525 for high versus low quality prey) was in the predicted direction but not statistically significant.
526 However, the observed positive feedback effects were overridden by other factors that shaped
527 foraging behaviour independent of current gizzard mass. In addition, consistent among-individual
528 differences in gizzard mass were observed following staple diet treatments. As the staple diet
529 treatments precluded the opportunity to exhibit diet selectivity, our results indicate not only that
530 state-behaviour feedbacks at best play a limited role in generating consistent among-individual
531 differences in gizzard mass, but further, that among-individual differences in gizzard mass can arise in
532 the absence of feedbacks between gizzard mass and diet choice. We suggest that among-individual
533 differences in early dietary experience may result in individual differences in developmental
534 trajectories of digestive physiology, and/or that social context may play a key role in mediating
535 foraging opportunities, which in turn determine gizzard mass. Further studies are needed to
536 experimentally assess the importance of these alternative mechanisms.

537

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559

560 **Data accessibility**

561 Data deposited in the Dryad repository: <http://dx.doi.org/10.5061/dryad.k28j0> (Mathot, Dekinga &
562 Piersma 2017).

563

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689

690 **Supporting Information**

691 Additional supporting information may be found in the online version of this article.

692 **Appendix S1:** The relationship between body size and gizzard mass

693 **Appendix S2:** Determining size categories of *Macoma* for use in experiments

694 **Appendix S3:** Determining optimal diet for digestively constrained foragers

695 **Appendix S4:** Comparing digestive constraints and optimal diet predictions across two studies

696 **Table S1:** Energy and ballast content for two size classes of *Macoma* used in the experiments

697 **Figure S1:** Solving for the optimal diet by the digestive rate model

698 **Figure S2:** Correlations between gizzard mass measurements

699 **Figure S3:** Behavior exhibited during foraging trials does not correlate with field gizzard mass

700 **Table 1:** Intake rate (number of *Macoma* eaten) during ad libitum trials, and probability of eating high and low quality *Macoma* as a function of gizzard
701 treatment and replicate. Intake was modelled with Gaussian errors and probability of acceptance (accept=1, reject =0) was modelled with binomial errors.
702 Values presented are estimates (β for fixed effects, σ for random effects) and 95% credible intervals around the estimates (CI) drawn from 1000 iterations of
703 the model (see methods for further descriptions of the models). Adjusted repeatability (r) was calculated as the individual variance divided by the sum of
704 individual and residual variance.

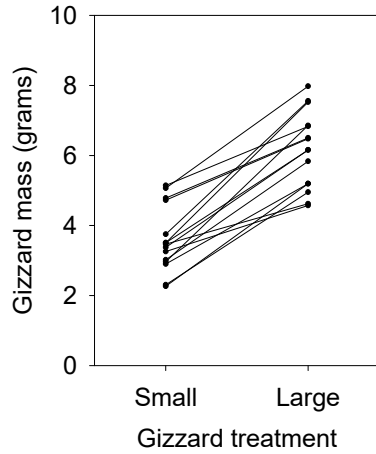
		Log odds ratio of accepting versus rejecting		Log odds ratio of accepting
	<i>Ad libitum</i> intake	High quality <i>Macoma</i>	Low quality <i>Macoma</i>	Large versus Small
Fixed effects	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
Small gizzard treatment	7.28 (3.64, 11.46)	2.21 (1.40, 3.43)	-1.14 (-2.00, -0.61)	-1.41 (-2.00, -0.96)
Replicate (small gizzard treatment)	6.02 (2.14, 8.59)	1.34 (0.09, 2.97)	0.61 (0.33, 1.10)	0.46 (0.08, 0.74)
Large gizzard treatment	14.72 (10.20, 18.23)	2.08 (1.11, 3.02)	-0.72 (-1.57, -0.17)	-0.95 (-1.62, -0.56)
Replicate (large gizzard treatment)	0.63 (-2.84, 3.65)	0.24 (-0.48, 0.98)	-0.06 (-0.63, 0.45)	-0.02 (-0.57, 0.39)
Random effects	σ (95% CI)	σ (95% CI)	σ (95% CI)	σ (95% CI)
ID	2.24 (0.75, 5.05)	0.93 (0.47, 1.94)	0.44 (0.22, 0.92)	0.12 (0.03, 0.19)
Residual	41.52(27.18, 64.36)	1	1	1
Repeatability	r (95% CI)	r (95% CI)	r (95% CI)	r (95% CI)
ID	0.05 (0.02, 0.09)	0.57 (0.36, 0.68)	0.36 (0.22, 0.50)	0.10 (0.03, 0.16)

705

706 **Table 2:** Gizzard plasticity (change in gizzard mass over the course of diet trials; gizzard mass at end
707 of diet trials – gizzard mass at start of diet trials) as a function of gizzard mass treatment (“small” or
708 “large”) and total number of *Macoma* consumed. Gizzard plasticity was modelled with a Gaussian
709 error structure. Values presented are estimates (β for fixed effects, σ for random effects) and 95 %
710 credible intervals around the estimates (CI) drawn from 1000 iterations of the model (see methods
711 for further descriptions of the models). Adjusted repeatability (r) was calculated as the individual
712 variance divided by the sum of individual and residual variance.

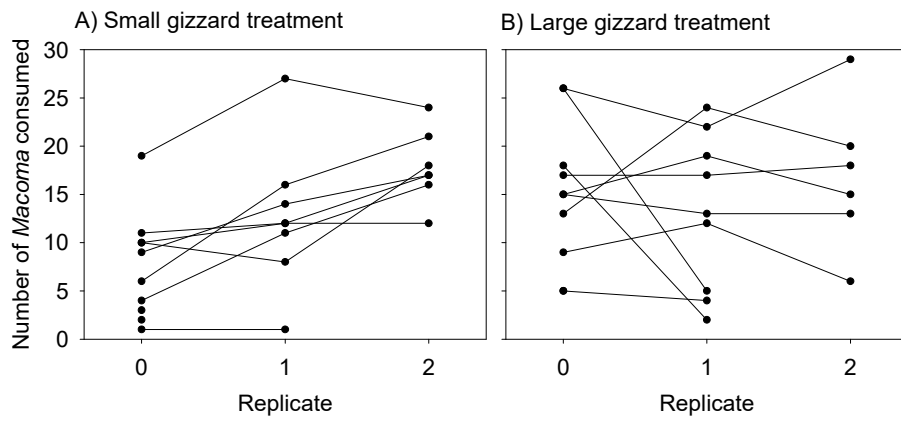
Fixed effects	β (95% CI)
Small gizzard treatment	-0.15 (-1.28, 0.69)
<i>Macoma</i> ingestions (small gizzard treatment)	0.019 (0.005, 0.037)
Large gizzard treatment	-1.42 (-2.45, -0.38)
<i>Macoma</i> ingestions (large gizzard treatment)	0.035 (0.014, 0.051)
Random effects	σ (95% CI)
ID	0.80 (0.34, 1.52)
Residual	1.16 (0.67, 2.05)
Repeatability	r (95% CI)
ID	0.43 (0.25, 0.58)

713



714

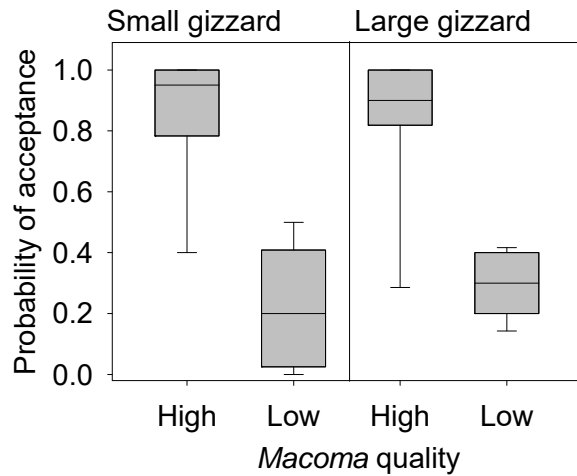
715 **Figure 1:** Effect of three week long gizzard mass treatment on realized gizzard mass at the start of
 716 diet choice experiments. Lines connect estimated gizzard mass from the same individual red knot in
 717 each of the two treatment categories. During the small gizzard treatment, birds received a high
 718 digestive quality diet of the flesh from open mussels, and during the large gizzard treatment birds
 719 received a low digestive quality diet of gastropod mudsnails, *Hydrobia ulvae*, which required
 720 processing large shell volumes in the gizzard.



721

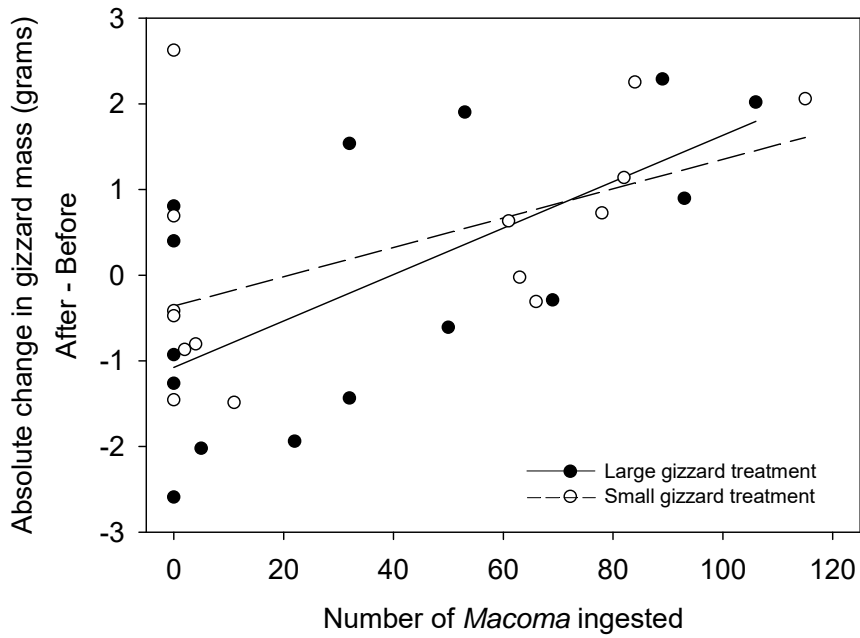
722 **Figure 2:** Effect of gizzard mass manipulations on the number of *Macoma* consumed during three

723 consecutive *ad libitum* foraging trials. Lines connect successive trials by the same individuals.



724

725 **Figure 3:** Probability of accepting high versus low digestive quality *Macoma* as a function of gizzard
 726 mass. Box plots illustrate raw data for first trial in which a bird participated for 4 or more prey
 727 presentations. Lines within the boxes mark the medians, boxes span the 25th to 75th interquartile
 728 range, and the whiskers indicate the 90th and 10th percentiles.



729

730 **Figure 4:** Changes in gizzard mass as a function of the total number of *Macoma* ingested during three
 731 foraging trials per bird. Each point represents 1 individual. Filled circles denote the large gizzard
 732 treatment and open circles denote the small gizzard treatment with opened mussels. Regression
 733 lines are best fits to the raw data.